MEASUREMENT OF 5 S RNA ACCUMULATION IN OOCYTES OF *XENOPUS LAEVIS* USING A cDNA PROBE

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1. Introduction

During oogenesis in amphibians such as *Xenopus laevis* there is an accumulation of 5 S ribosomal RNA which is not coordinated with the transcription of the larger ribosomal RNA species; oocyte-specific 5 S genes are switched on early in development and 5 S RNA accumulates as 7 S and 42 S particles complexed with protein(s) and, in the case of the 42 S, also with tRNA [1,2]. Only later in oogenesis is the 5 S RNA accomodated into ribosomes. The abundance of a 37 000-M_r protein factor has been implicated as necessary for the transcription of 5 S genes by RNA polymerase III in the regulation of these events. This factor is present at early times in oogenesis, has a preference for somatic rather than oocyte-type 5 S genes and is sequestered into the 7 S particle as 5 S synthesis proceeds [3–5]. We have become interested in 5 S transcription in very small (previtellogenic) oocytes, and report here the use of a 5 S cDNA probe to show that 5 S accumulation is most marked in the small oocytes of very young frogs and also that some individuals exhibit a precocious development of large oocytes which are depleted in 5 S RNA.

2. Materials and methods

2.1. *Materials*

Wild- and captive-bred *Xenopus laevis* were obtained from *Xenopus* Ltd (Redhill). [^{3}H]Deoxycytidine triphosphate was from the Radiochemical Centre (Amersham). AMV reverse transcriptase was obtained from Dr J. W. Beard through Program Resources and Logistics of the US National Cancer Institute. Polyester meshes were obtained from Henry Simon Ltd (Stockport). Unlabelled deoxyribonucleoside triphosphates, collagenase, hyaluronidase and S1-nuclease were from the Sigma Chemical Co. (Poole).

2.2. *Preparation of oocytes*

Ovaries were cut into small pieces and washed extensively in Barth X solution [6]. These were then incubated at 25°C with 2.5 mg collagenase/ml for 1–2 h until the oocytes were free of follicle cells and connective tissue. Sizing of oocytes was by passage through a series of polyester meshes as in [7]. The method was modified slightly when preparing oocytes from very young animals with the addition of 2.5 mg hyaluronidase/ml to the digestion step in buffer adjusted to pH 8 with NaOH.

2.3. *RNA isolation*

Total RNA was extracted from whole oocytes essentially as in [8] with 0.1% SDS in the aqueous phase. After ethanol precipitation, samples were incubated with 10 μg DNase I/ml (RNase-free) at 37°C for 15 min, re-extracted with phenol and reprecipitated with ethanol. ^{3}H-Labelled 28 S RNA was added to crude homogenate samples to monitor recovery, which was always >80%.

2.4. *5 S cDNA preparation and hybridisation*

cDNA was made as in [9]; hybridisation conditions were also as in [9]. $R_o t_{1/2}$ values of 5 S cDNA with pure 5 S RNA were in the region of 1.53×10^{-4} ($\pm 0.03 \times 10^{-4}$).

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3. Results

Oocytes were isolated from ovaries of female *Xenopus laevis* and separated into different size classes which correlate with morphological criteria [10]:

Class 1: 50–125 μm diameter;
small, clear, correspond to early Dumont stage I.
Class 2: 125–200 μm diameter;
clear, late Dumont stage I.
Class 3: 200–300 μm diameter;
mostly white (early vitellogenic), Dumont stage II.
Class 4: 300–500 μm diameter;
some white, many brown (Dumont stage III).
Class 5: 500–1000 μm diameter;
mixed Dumont stages IV–VI, in varying proportions.
Animals <4 months old contained mainly class 1 oocytes.

A counted number of oocytes from each size class was used for extraction of total RNA and this was hybridised to cDNA to give the appropriate R_0t curves. The results shown in fig.1 are typical of those found with mature, captive-bred animals and showed as expected that in early stages of oogenesis there was a high percentage of 5 S sequences (up to 22% of total RNA) which decreased quite sharply in class 3 cells, presumably reflecting the onset of 18 S and 28 S ribosomal RNA production. The proportion of 5 S RNA in large oocytes of these frogs was virtually as low (2%) as in somatic cells; in fig.1 the R_0t curves of class 5 oocytes and liver cells are very similar. A range of 5 S content from between ~15 ng/class 1 oocyte (~3×10^{11} molecules) up to 45–65 ng/class 3/4 oocyte ($\geqslant 10^{12}$ molecules) was routinely observed in mature frogs.

Fig.2 shows the effect of time after metamorphosis on the proportion of 5 S RNA in class 1 oocytes in a typical series of frogs of different ages. Six weeks after metamorphosis the percentage of 5 S RNA in class 1 oocytes was as low as in liver cells, but a dramatic increase had occurred by 3-months and levels were higher still after 8-months. Three-month-old animals had ~0.18 ng 5 S RNA/class 1 oocyte (7% of total and ~3×10^8 molecules) and 8-month animals had the full adult amount (21%, 3×10^{11} molecules).

We have noticed, however, that there are excep-

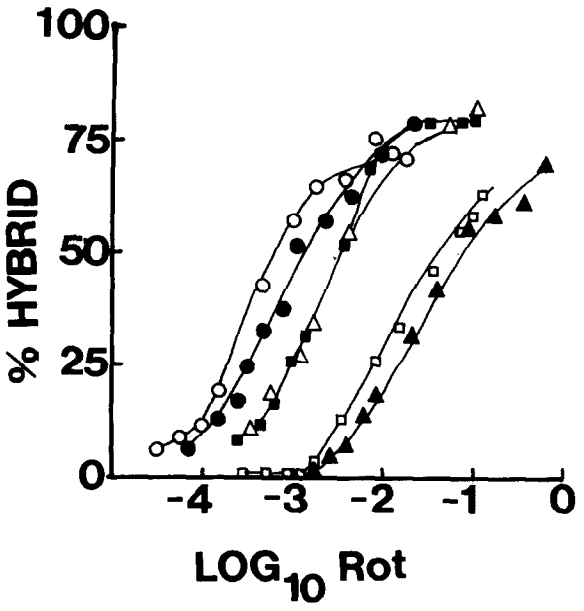

Fig.1. Hybridisation kinetics of 5 S cDNA and RNA from different-sized oocytes of a single mature frog (captive-bred): (○) RNA from class 1 oocytes; (●) RNA from class 2; (■) RNA from class 3; (△) RNA from class 4; (□) RNA from class 5; (▲) RNA from liver. Maximum hybridisation levels for calculation of $R_0t_{1/2}$ were taken as 80%.

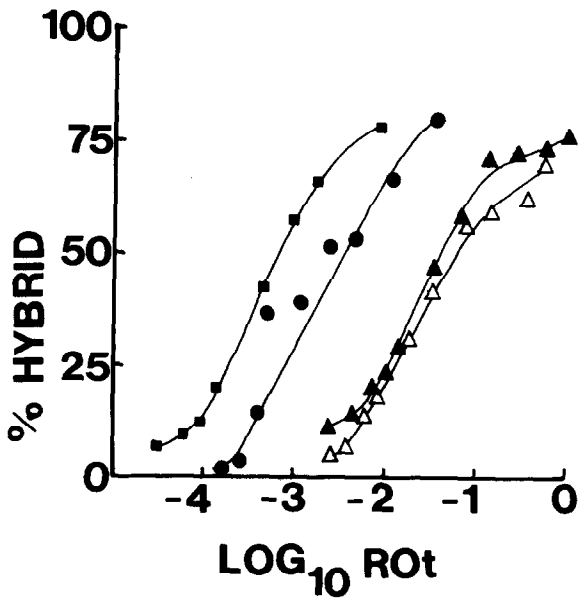

Fig.2. Hybridisation kinetics of 5 S cDNA and RNA from class 1 oocytes derived from frogs of different ages: (▲) oocyte RNA from 6-week-old animals; (●) RNA from 3-month-old animals; (■) RNA from 8-month-old animals; (△) RNA from adult liver.

Table 1
5 S accumulation in individual frogs

Frog	Oocyte size class	RNA content (ng/cell)		% 5 S RNA
		Total	5 S	
Wild adult	1 + 2	380	14.3	3.8
	3	380	28.5	7.5
	4	920	27.6	3.0
	5	2260	45.0	2.0
8-month (captive-bred)	1	70	15.0	21.4
	2	370	44.5	12.0
	3	430	64.5	15.0
	4	980	47.0	4.8
	5	4700	17.4	0.4
12-month (captive-bred)	1	5	0.6	12.0
	2	263	39.5	15.0
	3	n.d.	n.d.	n.d.
	4	1000	30.0	3.0
	5	2630	15.8	0.6

n.d., not determined

RNA was extracted from counted numbers of oocytes of the various size classes and estimated by absorbance at 260 nm. Amounts per cell were corrected for extraction efficiency using a [^{3}H]RNA marker (see text) and the RNA hybridised in excess to cDNA. Hybrid formation was determined after S-1 nuclease digestion by retention on DE 81 filter discs

tions to this pattern of 5 S accumulation during the first year of life. In table 1 the results from a wild adult are compared with those from atypical 8- and 12-month-old captive-bred individuals. In our experience wild adults often have rather few class 1 oocytes (hence 1 and 2 size-classes were usually pooled), but the 5 S content generally increased in total and decreased in proportion to the point expected (2%) as a function of increased oocyte size. The younger animals illustrate the observation that growth and development rates of ovaries vary substantially between individuals and this is reflected in different patterns of 5 S RNA accumulation. The 8-month-old frog had an unusually well-developed ovary with oocytes of all sizes, and with rather high amounts of total RNA/cell. However, the large class 5 oocytes had much less 5 S RNA than needed to maintain stoichiometry in the ribosomes, assuming that most of the rest consisted of 18 S and 28 S sequences as is usually the case. In this instance there would be only about one 5 S molecule/4 ribosomes. It is not possible from this kind of study to determine whether there has been degradation of 5 S RNA in the larger oocytes or whether their precocious development in some way reduced the rate of 5 S synthesis so that these particular cells never aquired the levels seen in class 3 oocytes from the same frog. The 12-month-old individual showed a similar aberration in the larger oocytes and in addition had accumulated very little RNA in its class 1 cells.

4. Discussion

The early and dramatic accumulation of 5 S RNA during oogenesis in *Xenopus* has been know for at least a decade [1]. More recent discoveries in this area have included the heterogeneity of amphibian oocyte-type 5 S sequences and a factor specifically involved in mediating 5 S gene transcription by RNA polymerase III. We set out to develop a specific and sensitive hybridisation probe with the capability of measuring 5 S content of small numbers of oocytes [9] and we describe here some uses of 5 S cDNA in this context.

The pattern of 5 S accumulation we have observed in oocytes from mature frogs is broadly in agreement with that in [11] summing radioactivity of 5 S RNA under gel peaks, though the relative amounts indicated by cDNA were rather lower. Our animals were not subjected to regular ovulation in the laboratory, and since the animals used were from different sources and the oocyte size classes examined not exactly the same, the similarities in overall patterns are probably more important than variations in percentage of 5 S at particular stages.

This study indicates that there is a very rapid rate of 5 S accumulation in very small oocytes in the 6–8-months following metamorphosis. Up to 30% of the 5 S molecules needed in the mature oocyte were accrued by cells <125 μm in diameter. The rate of 5 S gene transcription during this period must be very high, since the appearance of 3×10^{11} RNA molecules within 8-months of metamorphosis infers a transcription rate by RNA polymerase III averaging >30 nucleotides/s, assuming only one RNA polymerase can bind to a 5 S gene at a time and $\sim 10^5$ 5 S genes in the tetraploid oocyte nucleus. The larger amounts of 5 S seen in stage 3 oocytes of some 8-month-old frogs (table 1) infer rates 3–4-times higher still may be possible, though it might be that synthesis started prior to metamorphosis in these animals.

In conclusion, the 5 S cDNA probe has allowed us to investigate 5 S RNA content in small numbers of oocytes which would be difficult to quantify using more direct analytical techniques. This in turn has highlighted a rapid accumulation of 5 S RNA in the smallest oocytes of very young frogs as well as permitting the investigation of 5 S metabolism in atypical individuals.

Acknowledgements

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